ASD TECHNICAL REPORT 61-708

A COLORIMETRIC DETERMINATION FOR 1, 1 - DIMETHYLHYDRAZINE IN AIR, BLOOD, AND WATER

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DECEMBER 1961

AERONAUTICAL SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
UNITED STATES AIR FORCE
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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AERONAUTICAL SYSTEMS DIVISION
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FOREWORD

This study was supported by Project No. 7165, "Health Hazards of Materials and Radiation," Task No. 716501, "Evaluation and Control of Toxic Chemical Materials." The work was conducted during the period April to July 1961. The work was initiated by the Toxic Hazards Section, Physiology Branch, Biomedical Laboratory of the Aerospace Medical Laboratory.

ABSTRACT

A simple, rapid colorimetric procedure is described for measuring microgram quantities of 1, 1-dimethylhydrazine (UDMH) in blood and water. The method, with minor modification, has also been adapted for analysis of air samples. The report provides a calibrated range for analysis of 1-60 micrograms UDMH per milliliter fluid and of 2. 5-50 ppm in air; its' useful range may be considerably extended by manipulative dilution techniques. The test has additional, limited qualitative application to the analysis of urine.

PUBLICATION REVIEW

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INTRODUCTION

The use of 1,1-dimethylhydrazine (UDMH) as a propellant material has prompted numerous investigations into its toxic properties and its pharmacological effects. Studies within these areas have been considerably hampered by the lack of a satisfactory procedure for identifying UDMH in biological fluids and tissues.

Several methods have been proposed and used for the determination of various hydrazine derivatives (refs. 1-8). Choice of a particular technique seems to have been dictated primarily by individual needs, such as air sample analysis for industrial hygiene and toxicological research purposes or the detection of specific medicinal drugs in body fluids for therapeutic purposes. The most commonly used methods generally are based upon titration or oxidation-reduction principles and are as such nonspecific. The most sensitive of the techniques previously described for detecting UDMH has been that involving reduction of phosphomolybdic acid with the formation of molybdenum blue and subsequent colorimetric measurement (refs. 1, 3). Unfortunately, this method is completely invalid to use with biological fluids because of their naturally occurring reducing substances. More recently, Prescott et al. (ref. 6) have described the use of the "pyridyl" test for the detection of hydrazine (N_2H_4) in biological materials, but the test lacks a sufficient degree of sensitivity to detect microgram amounts of UDMH. Scardi and Bonavita (refs. 7, 8) have reported a simple colorimetric method for determining isoniazid in blood by measuring intensity of the yellow color complex formed when isoniazid is reacted with trisodium pentacyanoamino ferrate (TPF). This same color reagent when added to UDMH produces a definite red color complex, which is easily measured in the ordinary spectrophotometer found in most laboratories.

A simple and rapid procedure to determine quantitatively the presence of UDMH using trisodium pentacyanoamino ferrate is presented together with comments about its use in making an early, definitive diagnosis of UDMH exposure.

DETECTION OF UDMH IN AIR

Materials

Reagents:

Trisodium Pentacyanoamino-Ferrate-(TPF) Color Reagent:

Fisher Scientific Company, Cat. No. S-659, Fair Lawn, New Jersey. A 0.1 percent solution in distilled water prepared daily. It is not stable over extended periods of time in acid media, i.e., below pH 6.

Buffer Solution:

pH 5.4, 1.92 grams citric acid (crystalline) and 5.65 grams disodium acid phosphate are dissolved in water to a total volume of 2 liters.

Special Equipment:

B and D 100-ml syringe, fitted with restriction orifice so that it delivers a known volume per minute, by gravity (our volume was 3.33 ml/min.).

Polyethylene Bubblers:

Glass fritted scrubbers are unsatisfactory for collecting air samples. Polyethylene bubblers are consistently 100 percent efficient for the air flow and volume of scrubbing liquid used in this procedure.*

Vacuum pump for pulling air through bubblers with polyethylene connectors and a calibrated flowmeter for measuring a flow rate of 1 liter per minute.

Method

Sampling and Analytical Procedure:

Two liters of air are scrubbed in 20 ml of buffer solution at a rate of 1 liter per minute using a polyethylene bubbler. A 10-ml aliquot of the scrub solution is treated with 1 ml of the TPF color reagent. A BLANK, prepared simultaneously, consists of 10 ml buffer solution to which 1 ml of the color reagent is added. Both BLANK and UNKNOWN are allowed to stand for 1 hour with occasional agitation. The UNKNOWN colored solution is transferred to a Leitz cuvette and the optical density measured against the reagent BLANK, contained within a matched cuvette, and using a Leitz Photo Colorimeter equipped with a 540-mm filter (green). The remaining 10 ml of the scrub solution should be saved so that it may be diluted and analyzed in the same manner if the first aliquot shows off-scale readings.

* The use of two bubblers in series demonstrated that all concentrations of UDMH analyzed were completely recovered in the first bubbler.

Standardization and Validity of Method:

Optimum Conditions of pH:

Maximum color intensities are obtained when the pH of the total reacting solution is between 4 and 6. A citric acid-phosphate buffer of pH 5.4, chosen as the scrubbing medium, is satisfactory.

Color Stability:

Maximal intensity of color develops 50-60 minutes after the color reagent is added and remains unchanged for 3 hours, after which time the color slowly fades. The color intensity decreases 13-19 percent at the end of 24 hours. One hour is the preferred time for reading as it gives consistently reproducible results. The amount of color reagent prescribed is ample to react with 120 micrograms of UDMH.

Standardization of the Test:

The color development procedure was applied to various buffered dilutions of a standard UDMH solution to show that the amount of colored product formed with TPF is directly proportional to the amount of UDMH present. Concentrations corresponding to 2.5, 10, 20, 30, 40, and 50 ppm UDMH in 1 liter of air were calculated according to the following formula:

$$\text{mg/liter} = \text{ppm} \times \frac{\text{molecular weight}}{24450}$$

Therefore:

2.5	ppm	=	6.04 µg/liter
10	ppm	=	24.14 µg/liter
20	ppm	=	48.28 µg/liter
30	ppm	=	72.42 µg/liter
40	ppm	=	96.56 µg/liter
50	ppm	=	120.7 µg/liter

The calculated µg per liter UDMH was converted to µl by using the density factor of UDMH (0.7861). A stock solution of UDMH was prepared and microliter quantities were delivered by microburette to 10-ml aliquots of buffer solution. A BLANK of 10-ml buffer solution was prepared simultaneously. One ml color reagent was added to each concentration; they were mixed and allowed to stand for 1 hour. The optical density of each concentration was measured against the BLANK set at 100 percent transmittance in a Leitz photocolorimeter using the green filter.

Linearity is limited to the 0-25 ppm range. However, the useful range can be extended to 50 ppm by reading directly from a calibration curve rather than by calculating in terms of a known standard concentration. Although increasing the concentration of the color reagent results in improved linearity between 25 and 50 ppm, stability and reproducibility of color intensity are sacrificed. Therefore, 1 mg per ml TPF concentration is considered most satisfactory for this test procedure.

Recovery of Vaporized UDMH in Air Samples Scrubbed in Buffer:

Known amounts of UDMH were vaporized in a 100-ml syringe and metered through a critical orifice directly into the air stream of the polyethylene bubbler. The amounts of UDMH were chosen so that concentrations ranging from 2.5 to 50 ppm were produced. The results are shown in figure 1.

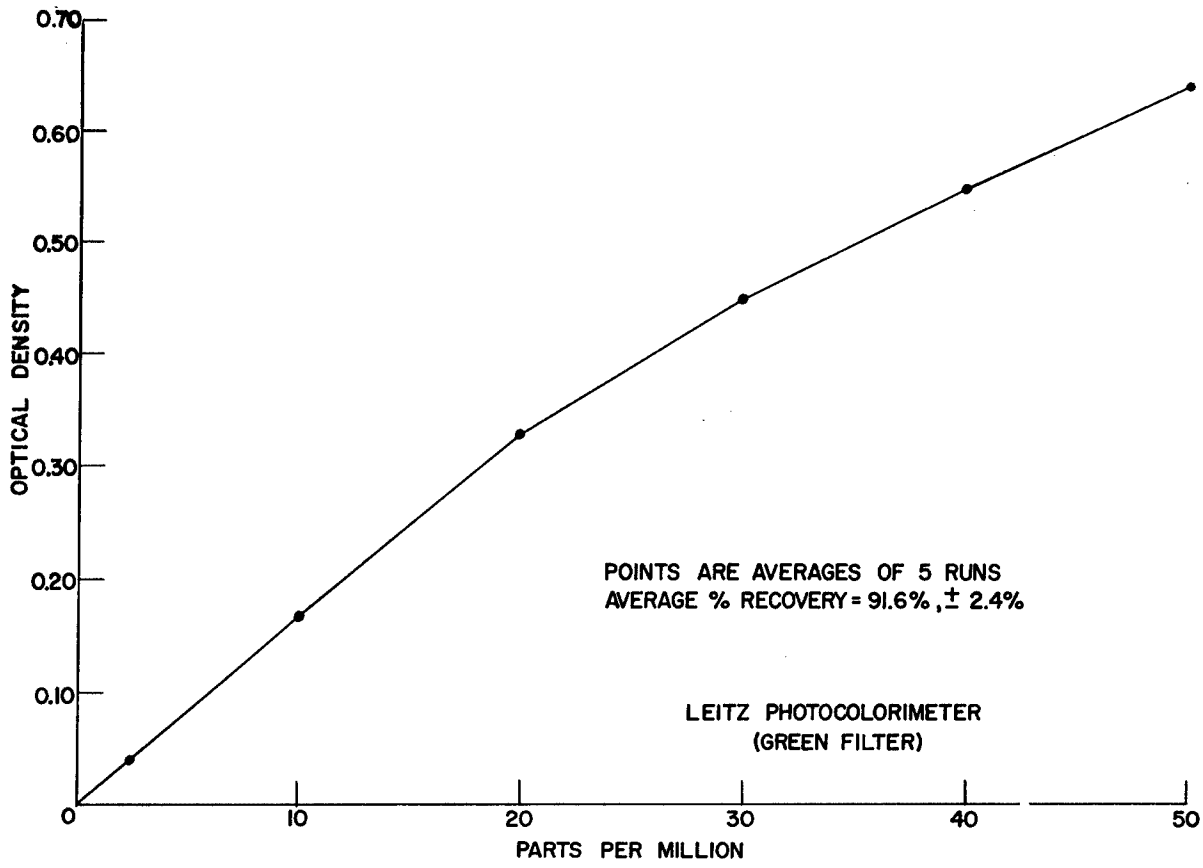

Figure 1. UDMH in Air Syringe Calibration Using TPF Reagent

Interference Tests:

Three thousand ppm UDMH was vaporized first with an equal concentration of hydrazine, then with an equal concentration of nitrogen dioxide (NO_2). The resulting mixtures were analyzed and compared with results obtained using an identical concentration of UDMH alone. The NO_2 reduced the color 20 percent. Dilutions of a 25 percent aqueous solution of dimethylamine did not form the red color complex with the color reagent.

DETERMINATION OF UDMH IN BLOOD

Materials

Reagents:

Buffer Solution:

pH 5.4; 9.6 grams citric acid and 29.25 grams disodium acid phosphate ($Na_2HPO_4 \cdot 7\ H_2O$) are dissolved in water to a total volume of 2 liters.

Sulfuric Acid:

0.667 N

Sodium Tungstate:

10 percent aqueous solution.

Trisodium Pentacyanoamino-Ferrate:

1 mg per ml water, prepared fresh daily.

Method

Calibration of Standard Curve:

Five, 10, 20, 40, and 60 µg quantities of UDMH, each contained in 10 ml of buffer solution, are reacted with 1 ml color reagent, and exactly 1 hour is allowed for full color development. All concentrations are read at 500 mµ in a spectrophotometer against a reagent BLANK set at 100 percent transmission. The BLANK consists of 10 ml buffer and 1 ml color reagent prepared simultaneously with all test procedures. Figure 2 shows optical densities plotted against concentrations.

Test Procedure:

A 1:10 protein-free filtrate is prepared using 1 ml of heparinized whole blood. Seven ml of distilled water is added to 'lake' the blood, followed in 3 minutes by 1 ml of 0.667 N sulphuric acid and 1 ml of 10 percent sodium tungstate. The sample is shaken vigorously and centrifuged for 15 minute at 2000 RPM.

Five ml of the water-clear supernatant is transferred to a cuvette. Five ml of citric acid-phosphate buffer is added.* The BLANK or reference cuvette contains 10 ml buffer solution. One ml color reagent is added to all cuvettes and the color is allowed to develop for 1 hour. The red-orange color is read at 500 mµ in a spectrophotometer against the reference tube set at 100 percent transmittance. Optical density readings are converted to micrograms by reference to the prepared calibration curve.

Calculation of Results:

µg x 2 = µg UDMH per ml whole blood

* If multiple samples are being analyzed simultaneously, all sample supernatants should be buffered before adding the color reagent to any cuvette.

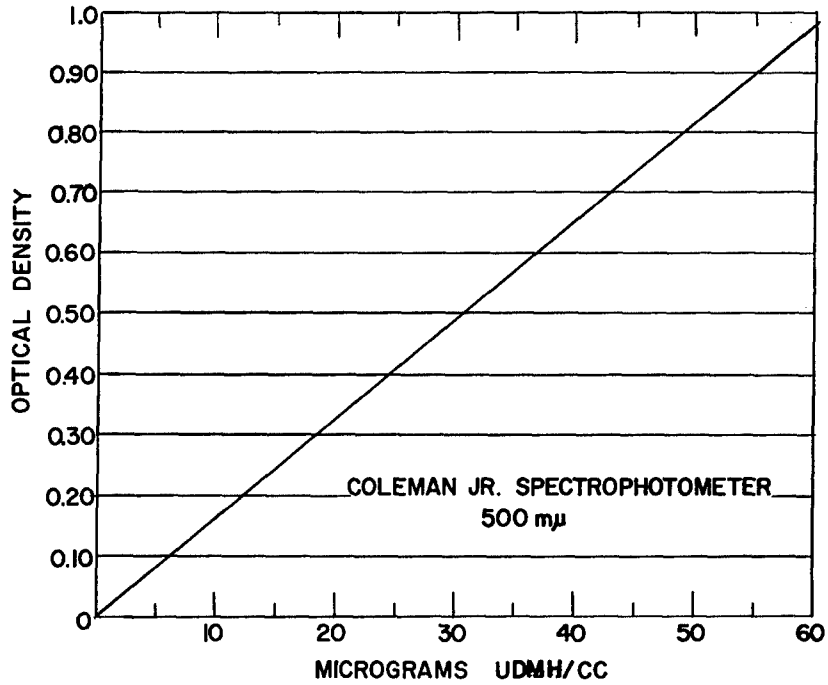

Figure 2. Calibration Curve for UDMH in Buffer

DETERMINATION OF UDMH IN WATER SAMPLES

Materials

Reagents:

Buffer Solution:

pH 5.4, prepared in the same concentration and in the same manner as described under the procedure for blood analysis (p. 5).

Trisodium Pentacyanoamino-Ferrate:

Prepared in the same manner and concentration as described under the procedure for blood analysis (p. 5).

Method

To 1 ml of the clear water sample, filtering if necessary to remove particulate matter, add 9 ml of buffer solution. A BLANK is prepared by using 10 ml of buffer solution with no reagent. To both the UNKNOWN and BLANK samples add 1 ml of color reagent. Allow samples to stand at room temperature for 1 hour to allow maximum color development. The BLANK is set at 100 percent transmittance in a spectrophotometer using a 500 mμ wave length. Read the optical density of the UNKNOWN red-orange color and convert to micrograms of UDMH using the same calibration curve as was constructed for blood samples. This can be done because water and buffer calibration curves are superimposable.

GENERAL DISCUSSION OF METHODS

Stability of the Color Reagent Solution:

The color reagent made up in water remains stable for 8 hours at ordinary room temperatures. However, to insure maximum accuracy, a BLANK solution should be prepared with each set of determinations.

Concentration of the Color Reagent:

Concentrations of TPF greater than 1 mg per ml produce unstable color complexes and cannot be used to prepare a standard calibration curve; i.e., peak intensity of the color develops and decays with increased rapidity as UDMH concentration increases.

Development of the Color:

Under the conditions of this test, the reaction between UDMH and TPF occurs slowly and is usually complete in 20-50 minutes. The color is stable for 1-2 hours at room temperature and then begins to decrease in intensity. At the end of 24 hours, the color fades 13-19 percent. Color intensity, stability, and development rate of the chromogen are dependent upon the pH of the final solution.

Influence of pH:

Although it is beyond the scope of this report to make detailed comments regarding various colored reaction products (refs. 2, 7, 8) which may occur in the presence of different hydrogen ion concentrations, we have found that the most reproducible and satisfactory results are obtained at a pH of 5.4± 0.5.

Absorption Spectra of the Reaction Product and the Color Reagent:

The product of the reaction between UDMH and trisodium pentacyanoamino ferrate shows a red-orange color with maximal and sharp absorption at 500 mμ. The color reagent is yellow and shows a maximal absorption at 400 mμ, presenting no interference at the 500 mμ wave length (figure 3).

Specificity:

To test the specificity of the color reaction between UDMH and trisodium pentacyanoamino ferrate, several substances somewhat similar in structure were examined under the same conditions, and in the same concentration ranges. Materials tested were hydrazine, nitrosodimethylamine, dimethylamine, and urea. None of these gave the characteristic red color complex which was obtained with UDMH. No color is developed with deproteinized serum or whole blood on addition of the color reagent; therefore, no correction is necessary for blanks.

Recovery of UDMH from Water, Air, and Blood:

Recovery experiments were performed by adding UDMH in amounts ranging from 5 to 60 micrograms per ml to serum, whole blood, water, and air. We recovered 100 percent from serum, whole blood, and water. Air samples, using the syringe metering technique and scrubbed through buffer solution, were 90 percent recovered (average recovery over the entire range of 2.5 to 50 ppm). The 10 percent loss expressed here is thought to be the result of adsorption on the glass surface of the syringe, a purely artificially created condition which would not be expected in a practical monitoring situation.

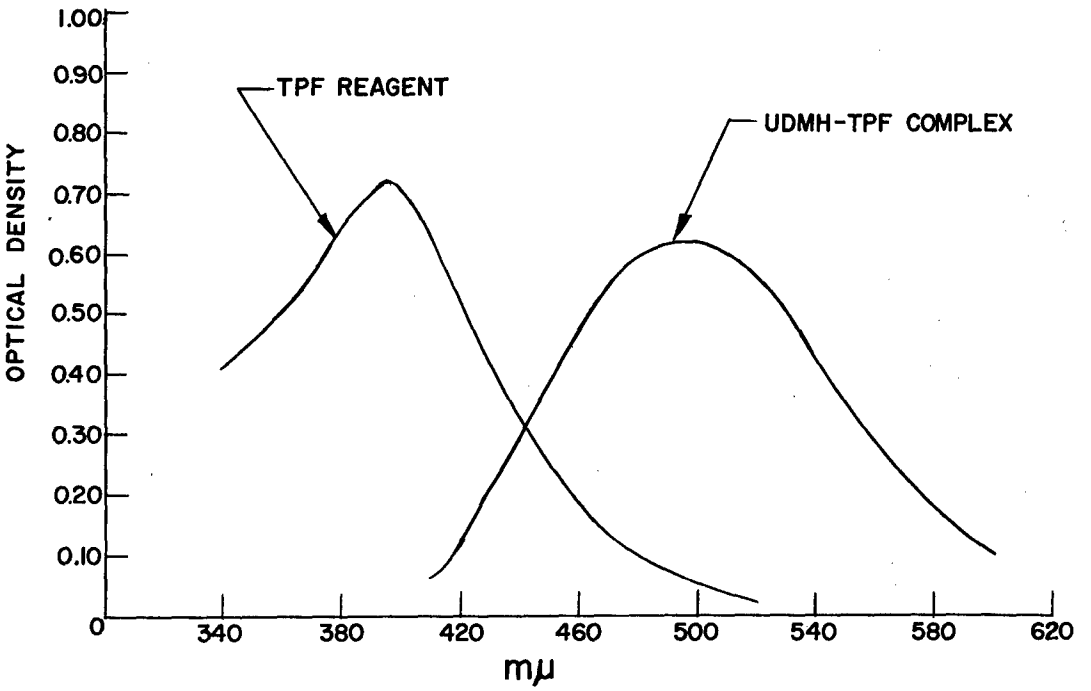

Figure 3. Absorption Spectra

RECOVERY OF UDMH FROM URINE

Addition of UDMH to urine samples in amounts ranging from 5 to 50 μg per ml did not result in reproducible or predictable recoveries. Neither were the results consistent from individual to individual nor from time to time. Inhibition of the color complex ranged from 0 to 100 percent in some samples, and all efforts to remove inhibitory factors were unsuccessful. Rather perfunctory experiments indicated that the inhibition was the result of some action with the color reagent rather than with the UDMH. Related experiments performed in this Laboratory have shown that diuresis and catheterization of animals exposed to UDMH produces urine specimens from which 100 percent recoveries may be consistently obtained. Despite the erratic recovery results, which make the exact quantitation of UDMH in urine impractical, valuable qualitative information may yet be provided in certain instances. Since we have experienced no instances of 'false positives' in our Laboratory, any positive results obtained in a urine sample would be almost conclusive evidence of a true exposure to UDMH.

CONCLUSIONS

A simple, rapid colorimetric procedure has been developed for the quantitation of microgram quantities of 1, 1-dimethylhydrazine in blood, air, and water. The method has a high degree of specificity and provides a significant increase of sensitivity over previously reported methods. This procedure utilizes equipment and reagents that are common to most laboratories and can be performed with a minimum of time and specialized technical knowledge. Analysis of blood and urine using this colorimeter procedure can provide valuable information for the early diagnosis of UDMH exposure cases.

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Preparation of Sodium Pentacyanoaminoferroate (in lieu of using Fisher's reagent)

Ten grams of finely powdered sodium nitroprusside are treated in a flask with 32 ml of concentrated ammonium hydroxide overnight, at refrigerator temperature. Absolute ethanol is added to the mixture and a yellow precipitate is obtained. The precipitate is washed with absolute ethanol and anhydrous ether until dry. The resulting sodium pentacyanoaminoferroate must be stored in a dessicator.

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Unclassified report.

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